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**CRISPR/Cas9 microinjection in oocytes disables pancreas development in sheep.**

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**Public Summary:**

The human organ transplant waiting list is long. A major goal in regenerative medicine is to generate a stable supply of organs for transplantation that does not rely on human organ donation. For example, a pancreas transplant can cure patients with type-1 diabetes. We have previously shown that complex solid organs can be efficiently generated from iPSCs by a process called interspecies organogenesis. We ultimately want to generate human organs in large animals such as sheep. However, for efficient human organ formation, we must block the sheep organ from being formed. This paper represents an important step towards this goal by genetically block pancreas development in sheep.

**Scientific Abstract:**

One of the ultimate goals of regenerative medicine is the generation of patient-specific organs from pluripotent stem cells (PSCs). Sheep are potential hosts for growing human organs through the technique of blastocyst complementation. We report here the creation of pancreatogenesis-disabled sheep by oocyte microinjection of CRISPR/Cas9 targeting PDX1, a critical gene for pancreas development. We compared the efficiency of target mutations after microinjecting the CRISPR/Cas9 system in metaphase II (MII) oocytes and zygote stage embryos. MII oocyte microinjection reduced lysis, improved blastocyst rate, increased the number of targeted bi-allelic mutations, and resulted in similar degree of mosaicism when compared to zygote microinjection. While the use of a single sgRNA was efficient at inducing mutated fetuses, the lack of complete gene inactivation resulted in animals with an intact pancreas. When using a dual sgRNA system, we achieved complete PDX1 disruption. This PDX1(-/-) fetus lacked a pancreas and provides the basis for the production of gene-edited sheep as a host for interspecies organ generation. In the future, combining gene editing with CRISPR/Cas9 and PSCs complementation could result in a powerful approach for human organ generation.

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